Final report

for the CORE Organic Cofund funded project

"Refined forage legumes as local sources of protein feed for monogastrics and high quality fibre feed for ruminants in organic production"

Period covered: 1 May 2018 – 1 November 2021

Press juice of red clover and leaf stripper fraction of lucerne

Content

1. General information

1.1 Project information

Project information			
Project acronym	ProRefine	**Project ID**	2451
Project title	Refined forage legumes as local sources of protein feed for monogastrics and high quality fibre feed for ruminants in organic production		
Project website	https://projects.au.dk/coreorganiccofund/core-organic-cofund-projects/prorefine/ https://www.nibio.no/en/projects/prorefine?locationfilter=true		
Details of the project coordinator			
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Institution	Norwegian Institute of Bioeconomy Research (NIBIO)	**Country**	Norway
Start of project	1 May 2018	**End date of project**	31 April 2021
Duration in months	36	**New end date in case of a project extension due to COVID-19**	1 November 2021

1.2 Consortium

Partner no.	**Country**	**Institution/ organisation name**	**Type of institution/ organisation[1)]**	**Functions[2)]**	**Involved in WPs**	**Contact person[3)]**
P1	Norway	Norwegian Institute of Bioeconomy Research (NIBIO)	Public research centre	PC, WL, P	All	Steffen Adler, steffen.adler@nibio.no
P2	Italy	Università Cattolica del Sacro Cuore (UCATT)	University	WL, P	WP2, WP4, WP5	Paolo Bani, paolo.bani@unicatt.it
P3	Turkey	International Agricultural Research and Training Center (IARTC)	Public research centre	P	WP2, WP4, WP6	Ülfet Erdal, ulfeterdal@yahoo.com
P4	France	Trust'ing – Alf'ing	Company	P	WP2, WP3, WP4, WP5, WP6	Eric Juncker, ecpaval@hotmail.fr
P5	Norway	Ruralis - Institute for Rural and Regional Research	Private research centre	P	WP2, WP3, WP2, WP6	Brit Logstein, brit.logstein@ruralis.no

P6	Sweden	Swedish University of Agricultural Sciences (SLU)	University	WL, P	WP2, WP3, WP4	David Parsons, david.parsons@slu.se
P7	France	Institut National de la Recherche Agronomique (INRAE)	Public research centre	P	WP2, WP5	David Renaudeau, david.renaudeau@inrae.fr
P8	Denmark	Aarhus University (AU)	University	WL, P	WP2, WP4, WP5	Søren Krogh Jensen, skj@anis.au.dk

1) University, Public research centre, Private research centre, Company, Other
2) PC = Project coordinator, WPL = Work package leader, WPCL = Work package co-leader, P = Participant
3) Inclusive e-mail address

2. Summary

2.1 Final project summary suitable for web publication for a wider audience

The aim of the research project ProRefine was to improve local production of protein feed in organic production, in particular for monogastrics, in different regions in Europe and Turkey, through improved forage processing. The processing methods studied were leaf stripping and juice pressing of forage legumes (Figure 1). Both methods produce protein-rich fractions with high digestibility suitable for monogastrics and fibre-rich fractions suitable for ruminants.

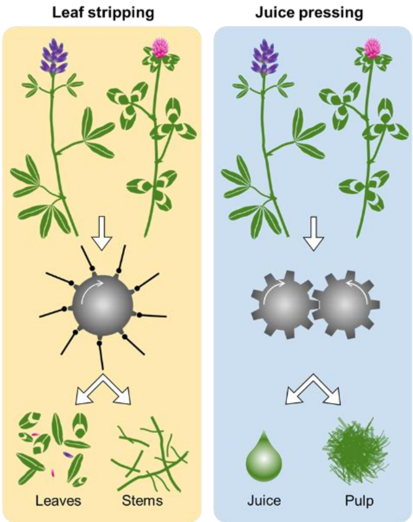


Figure 1. A leaf stripper can process forage legumes into a protein-rich leaf fraction and a fibre-rich stem fraction, and a twin screw press can process forage legumes into a protein-rich juice fraction and a fibre-rich pulp fraction (Illustration Steffen Adler).

The project addressed this through field experiments with different forage legume species in Sweden, Norway and Turkey, development of mathematical models for protein supply from forage legumes, feeding experiments with pigs in France and lambs in Italy to evaluate the feed value of protein- and fibre-rich fractions produced in Denmark, conceptualisation of local value chains based on forage legume fractionation, in depth and focus group interviews with farmers and stakeholders in the value chain, and assessment of social, economic and environmental aspects of sustainability.

In Turkey two varieties of lucerne were compared and harvested and processed seven times in 2019. At first harvest we found on average 43% of the dry matter yield in the juice fraction and when using manual leaf stripping, we found 53% of the dry matter yield in the leaf fraction. Mean crude protein (CP) concentrations in whole plant, juice, leaves, and leaf juice were 18%, 23%, 25% and 26%, respectively. This indicates that both methods can be used to produce fractions with increase CP concentration, and combination of both methods may give an additional, but rather small effect. In Sweden and Norway red clover gave higher yields than lucerne and were harvested and processed 3 to 4 times. The electric experimental leaf stripper developed in the project (PremAlfa Mini, Trust'ing – Alf'ing) also performed well in mixed stands of clover and grass. Results for mass balances, chemical composition and in vitro digestibility of the fractions are available in draft report and papers and will be published as a part of a PhD thesis. The methods (leaf stripper and screw press) produced products that were significantly different to each other and to the pre-harvest sward. The protein fractions (juice and leaf) had similar CP concentrations – in some cases the juice concentration was lower, and in some cases higher. The NDF concentration of protein fraction was always higher in leaves than juice – this is a key determinant of how these protein fractions can be utilised for livestock.

A full-scale leaf stripper (MRF2, Trust'ing – Alf'ing) and the pilot biorefinery plant at Aarhus university were used to fractionate lucerne and red clover crops. Leaves were mixed with barley meal and ensiled, stems were ensiled after short wilting period, press juice was precipitated to a protein concentrate and pulp was ensiled. The feeds were shipped to France and Italy. In fattening pigs, protein concentrate was assessed to be a good protein source, whereas leaf silages can be considered more an energy source. Especially in lucerne leaves, degradation of protein during fermentation was extensive. Ensiling experiments showed that use of additives can further improve the quality of preserved products.

Silages of stems and pulp had undergone a strong acetic fermentation process but were well accepted and consumed by lambs in the feeding experiments. Diets containing pulp silage resulted in better growing performance than diets containing stem silage, but both were considered valuable feeds for ruminants.

Implementation of leaf stripping can be done at farm level. For the production and stabilisation of press juice and inclusion in feed rations involvement of the feed industry is necessary. Currently, dairy farmers are sceptical to sell forage-based protein from their farms. The assessment of concepts and models of local food systems showed that animal productions with monogastrics can benefit from forage legume fractionation and cooperation by increase the level of self-sufficiency in feed. Whether dairy farmers have economic benefits depends highly on investment cost, production costs and price of protein concentrate. Estimates of Net Present Value (difference between the present value of cash inflows and the present value of cash outflows over a period of time) suggest that implementation of both fractionation methods can be feasible. Possible environmental benefits are related to less import of protein feeds and local protein production based on perennial forages.

The design of local food systems must be adapted to regional environmental conditions for plant and animal production, structures in agriculture and the views and attitudes of farmers and stakeholders. Preliminary results from the ProRefine project have been disseminated through stakeholder group meetings, conference contributions, newsletter and field days. Several manuscripts of scientific articles have been prepared and others are planned to be published in the near future.

The project has contributed with new knowledge about fractionation of forage legumes into feeds for animals from different species with different requirements. The project has also contributed with new knowledge about concepts of local food systems and actors' thoughts about self-sufficiency in feed in different regions. The project has also contributed with documentation of knowledge gaps that must be addressed in future projects.

2.2 Process update of the whole project

ProRefine achieved all its main objectives, however, we had to adapt the project plan several times due to unexpected situations.

The original plan included field experiments in each participating country to explore regional differences. The experiments in Sweden, Norway and Turkey were established according to the plan, but the experiment in France was abandoned in 2018 and in 2019 due to drought. We decided to produce all experimental feeds for the feeding experiments in Denmark and thus decided not to establish a plot experiment in Denmark. This was approved by the CORE Organic monitoring person and reported at the mid-term review. In brief, for best possible comparison of forage legume fractions in animal feeding experiments, we wanted to produce all feeds at the same location even though the planned feeding experiment with pigs was carried out in France and the planned feeding experiment with dairy cows was carried out in Italy. A leaf stripper (Trust'ing – Alf'ing) was shipped to Aarhus University, where a pilot-size biorefinery is also located. Leys with lucerne and red clover were harvested and processed, preserved and shipped to France and Italy.

Leaf stripper prototype MFR1 (Trust'ing – Alf'ing) and the electric PremAlfa Mini developed by the same company in the ProRefine project for experimental use. Photos: Trust'ing – Alf'ing and Anne de Boer, NIBIO.

Leaf stripping was done in Umeå, Sweden (left) with the PremAlfa Mini (Trust'ing – Alf'ing) and manually in Menemen, Turkey (centre). A tabletop juicer (Angel 7500, Korea) was used at all sites to separate press juice from pulp (right). Photos: SLU, Ülfet Erdal, Steffen Adler.

Trust'ing – Alf'ing developed an electric experimental leaf stripper (PremAlfa Mini) to be used in the plot experiments. Due to technical challenges, the machines were delayed and in 2019 could only be used in the third harvest in Sweden. Therefore, we decided to harvest the fields in Sweden and Norway for an additional season in 2020. Unfortunately, this winter in Northern Sweden was unfavourable for the overwintering of forage legumes and only some plots could be hand harvested to collect data for the modelling work. In Norway, lucerne did not overwinter, but three harvests of red clover and alsike clover were completed. The budget in Turkey did not allow to use the PremAlfa Mini and therefore leaf stripping was carried out manually.

The ProRefine project included a significant amount of transnational cooperation and therefore Covid restrictions stopped or delayed many activities or made them more laborious. These activities included shipping of samples, running feeding experiments and analysing samples, carrying out meetings, and collecting data. We applied for an extension and were granted 6 months extra time. This allowed us to complete most of the activities, however, most in-person dissemination activities had to be cancelled. Instead of a final project meeting we prepared voice-over presentations and made them available on the project websites. The project group will continue the dissemination work by publishing manuscripts as soon as they are ready for submission.

In the ProRefine project we have generated new knowledge on fractionation of forage legumes in different regions, assessed feed value of forage legume fractions, developed models for prediction of protein supply, developed concepts of local food systems, explored farmers' and stakeholders' views on self-sufficiency in feed, and assessed aspects of sustainability in models of value chains based on forage legume fractionation. The objectives for dissemination were only partly achieved due to the mentioned difficulties, but the list of manuscripts illustrates what we plan to publish in the near future.

3. Outcomes of the project

3.1. Main results, discussion, conclusions and fulfilment of objectives

WP1	*Project management*
WP leader: Steffen Adler **Responsible partners:** NIBIO and all partners	
Overall summary of main results, discussion and conclusions of WP1 This work packages included project management. Scientific work was carried out in WP2-6.	
Report on the results obtained (A), and fulfilment of objectives (B) comparing to the original project proposal	

The project group met for kick-off and mid-term meetings. The planned final meeting could not be carried out. Instead, we had web-based project meetings where possible and prepared voice-over presentations and made them available at the project webpages. The project coordinator participated in CORE Organic Cofund research seminars, one physical and one online. In addition, the coordinator visited the partners IARC, SLU, AU and Ruralis to discuss project work.

WP2	*Dissemination and industry engagement*

WP leader: David Parsons
Responsible partners: SLU and all partners

Overall summary of main results, discussion and conclusions of WP2

Diverse stakeholder groups were formed, and these were influential in the early months of the project in increasing the profile of the project, getting useful feedback from different stakeholders, and directing the research. The stakeholder groups also provided important input into WP6. The processes involved in this work package changed due to limitations imposed by the pandemic. Each country held either one or two stakeholder group meetings. Some later meetings were held virtually, however the stakeholders had less interest in such meetings compared with a physical meeting. To compensate for the reduced number of stakeholder meetings we focused on other dissemination methods.

Field days were also an important planned component of the project; however, these were only possible in two countries. The field days included discussion of the project, presentation of some results, and demonstration of equipment and techniques. A video recording of the Norway field day will be available as a project output after the video editing work has been finished.

There were a number of disruptions in the project, which caused delays in the generation and interpretation of results. This meant that materials for dissemination were completed later in the project than planned, and some are still in the process of being designed. Therefore, it is difficult to judge the response of the different stakeholders to the dissemination. Nevertheless, particularly in the final months of the project, a variety of dissemination materials were produced, and will provide a good record of the project, suitable for various stakeholders including farmers, advisors, and others.

Report on the results obtained (A), and fulfilment of objectives (B) comparing to the original project proposal

A- results obtained and structured in relation to the user groups they are relevant for:
The aims of this work package were: 1) To support technical and conceptual development and assessment of local food systems based on forage legume fractionation through a multidisciplinary (including agronomy, technology, and sustainability) and participatory approach. 2) Dissemination of promising results to farmers, advisory services, industry and government officials. 3) Generate knowledge that can be applied at a regional level according to differences in climate, agricultural structure, and social aspects by utilising coordinated regional activities with a common design.

The progress and results are as follows:

A2.1-4 Stakeholder group meetings
Stakeholder groups in were established in each region/country. The groups included a range of actors including government officials, farmers of different kinds of livestock, agri-business representatives, farm advisors, and veterinarians. The first stakeholder meetings included an introduction to the idea of bio-refining and the project, and sought feedback from the stakeholders. This included their ideas regarding project and experimental design, to adapt as much as possible to regional conditions. Having a focus on common design, feedback on the data collection process, and mapping perceptions and identifying potential issues regarding local feed systems was important. A questionnaire was developed for discussion with the stakeholders. These conversations were inputs for WP6 and are discussed in more detail there.

Most countries were able to have a second stakeholder group meeting. Subsequent stakeholder meetings, including final meetings, were not possible due to the effects of the pandemic. In general, the stakeholder meetings were well received. Participants were interested in the project, gave useful opinions, and expressed their views when they had reservations.

A2.5 Hosting of field days

Field days were planned for the third year, to practically demonstrate results of the project and reach a broader audience. Due to pandemic restrictions, they were only possible in Norway and Turkey. The field days included discussion of the project, presentation of some results, and demonstration of equipment and techniques. A video of the field day in Norway is available as a project output for download.

A2.6 Development of information for dissemination

There were various avenues for dissemination from the project, with outputs in multiple languages. Information sheets were published as practice abstracts, Core Organic fact sheets and other formats. Articles were published in the Core Organic Newsletter and other local publications, such as *Vallbrev*, the newsletter of the Swedish Grassland Society. Lists of dissemination activities and outputs are in Section 4. Some of the outputs are relevant for specific user groups. For example, the practice abstracts on ensiling are more oriented towards farmers and farm advisors. Most of the outputs are broader in their audience, and are relevant for farmers, operators in the feed industry, potential entrepreneurs, advisory services, and even the general public.

B- fulfilment of objectives:

The objective was to disseminate, with a participatory approach, innovative and locally sourced cropping and feed processing, including an assessment of sustainability of concepts of local food systems. This objective was partially fulfilled. The planned participatory approach was mostly not possible, due to the pandemic, except at the beginning of the project. Stakeholder groups were formed, however in most cases we were not able to meet with the groups again in person. Field days were only possible in two countries, also due to the pandemic. As a replacement, video presentations were planned. Three of these are available (https://www.nibio.no/en/projects/prorefine/dissemination?locationfilter=true), and a fourth will be published soon. In addition, there were many other avenues for dissemination including information sheets, newsletters, and presentations.

WP3	*Prediction of protein supply from forage legumes*

WP leader: David Parsons

Responsible partners: SLU and NIBIO, INRAE, Trust'ing – Alf'ing, UCATT, IARTC

Overall summary of main results, discussion and conclusions of WP3

The WP aimed to develop mathematical models to predict leafiness, CP content or fibre fractions of forages under different climatic conditions, thus informing management for efficient fractionation of forages. To do this, we collected climatic, morphological, and spectral data from various sources in Sweden and Norway, and used these data to develop prediction models, focusing on the bio-refined fraction.

The multiple regression models based on combinations of climatic, time-related, or morphological data, were generally not encouraging. Because the field experiments were primarily designed to harvest all plots on the same day, the climatic and time-related data were not particularly useful. However, a dataset using a leaf stripping machine in mixed stands developed good models for the CP and NDF concentrations of the leaf stripper fraction (LSF), utilizing a time-related variable (day of year) and morphological variables, combined with a handheld sensor such as a GreenSeeker. This mixed input data approach has potential for further development.

Sensor-only measurement using a spectroradiometer is another option for predicting forage quality and yield. Devices with many spectral bands that measure the whole canopy pre-harvest have potential for predicting CP, NDF, and CP yield. Currently this equipment is expensive and can be difficult to use in a repeatable way; however, increasingly devices are being commercialized, and they are likely to become cheaper and more practical to use.

The results show that it is difficult to estimate potential quality of the refined fraction based on measurements performed on the pre-harvest sward. In the near future, it is likely that harvesting decisions for bio-refining processes will be made based on factors like standing biomass, rather than predicted quality.

Report on the results obtained (A), and fulfilment of objectives (B) comparing to the original project proposal

A- results obtained and structured in relation to the user groups they are relevant for:

Activity 3.1 involved developing models for predicting lucerne and red clover yield, leafiness and concentration of CP. In addition to morphological data we also collected spectral data to address the objective.

We could not find any datasets outside of the Prorefine project that were useful for this activity. Thus, we had to rely entirely on data collection within the project. The forage experiments in France were abandoned due to drought and were not used for data collection. Therefore, the data collection for this activity focused on three sources: 1. Two production years of a field experiment in Sweden with five cultivars and two bio-refining methods. 2. Two production years of a field experiment in Norway with four cultivars and two bio-refining methods. 3. One production year of sampling from mixed stands of grass and clover in Sweden, using a leaf stripping machine.

For the field experiments, the data that were collected included the day of year, accumulated growing degree days above five degrees (GDD5) since the previous harvest, phenological development stage, canopy height, and tallest height. At both sites, two leaves per plot were assessed with a Linksquare portable contact spectrometer. In addition, in Sweden plots were scanned with a GreenSeeker and a Fieldspec 4 spectroradiometer.

When using the leaf stripper in mixed stands, the data that were collected included the day of year, clover phenological development stage, tallest clover height, GreenSeeker, Dualex, Linksquare, and Yara N-Sensor.

The main response variables included different combinations of forage fraction (whole plant, bio-refined fraction, residual fraction) and forage quality analysis (CP, CP yield, NDF, digestibility). Analysis of the data focused on stepwise multiple regression models for the morphological data, and partial least squares regression (PLSR) for the spectral data. The GreenSeeker and Dualex data give single outputs rather than spectra, so they were included in multiple regression models. The results for data sources 1 and 2 are in a draft report entitled “Models for predicting quality of bio-refined forages” (Chapter 4.5: A3.1. Parsons et al., in preparation). The results for data source 3 are in a draft scientific publication entitled “Testing a leaf stripping machine in mixed leys of grass and clover” (A4.7. Parsons et al., in preparation).

Field experiment datasets – the multiple regressions were run separately for Sweden and Norway, due to the differences in the datasets. There was insufficient data to estimate the quality of the LSF. There was no significant combination of explanatory variables that could predict the quality (either CP or NDF) of the juice fraction. Similarly, for the whole sward there were no significant models for NDF. The only significant models were for the whole sward CP concentration: r^2=0.56 for Sweden (based on day of year and GDD5) and r^2 =0.60 for Norway (based on day of year and tallest plant height). These results do not provide encouraging evidence that multiple regression models based on morphological measurements and weather data are possible. However, the design of the experiments necessitated that all plots were harvested at the same time for each harvest. This type of data does not lend itself well to development of regression models based on GDD or day of year, which have the same values for every plot at each harvest.

The second technique used to assess the field experiment data was the Fieldspec 4, which measures the reflected light from the canopy between 350 and 2500 nm at high spectral resolution. Analyses were done separately for the *Medicago* and *Trifolium* species, and for the leaf stripper fraction (LSF), juice and whole sward fractions. There were not enough data to get reliable results for the LSF. For the juice fraction, there was no significant PLSR model that could be developed to estimate CP concentration. However, CP yield (kg CP ha^{-1}) of the juice fraction could be explained with r^2 =0.90. For the whole sward, models were better for *Medicago* species than *Trifolium* species for both CP and NDF.

Mixed stands dataset – the most important output variable for the mixed stands data is the CP concentration of the LSF. A multiple regression model with r^2=0.76 was constructed by using the following variables: GreenSeeker, day of year, clover stage, and tallest clover. It is unsurprising that GreenSeeker was a useful variable because NDVI increases with greenness and with increasing biomass. The other variables in the model for CP are related to increasing time, plant size, or plant maturity, which are all correlated. A model to estimate the NDF of the LSF with r^2=0.87 included the variables day of year, Dualex sensor, tallest clover, and clover stage. In general, the multiple regression models for CP and NDF were quite good, and further investigation could explore whether they are repeatable methods for pre-harvest predictions of the quality of the LSF. Neither the Dualex or the GreenSeeker were useful for predicting CP or NDF when used on their own, without other inputs.

The second method to assess the mixed stands data was using the Yara N-Sensor, which measures the light reflected off the canopy between 400 and 1000 nm. The PLSR models for the LSF gave r^2 values of 0.90 for CP and 0.60 for NDF. Again, these models are promising for pre-harvest estimation of the quality of the LSF, particularly for CP. The Yara N-Sensor used was a handheld version of a commercialized tractor mounted device that is used for fertilizer recommendations.

B- fulfilment of objectives:

This WP developed models to predict the pre-harvest quality or protein supply of forage legumes; however, the usefulness of these models is variable. In general, the methods based on climatic, time-related, or morphological data did not explain enough of the variability between samples, and it is unlikely that they can be developed into reliable practical tools for farmers.

Because of this, we also tested a range of spectral equipment, that varied in area of effect (whole canopy or contact sensor) and the depth of spectral information (from two to hundreds of wave bands). We found that sensors that measure the whole canopy and have detailed spectral information are required. Unlike morphological models, spectral models also have the potential to incorporate biomass, to enable estimation of outputs like CP yield. Further development and testing is required before these models can be practically used.

WP4	*Upgrading forage legume crops*

WP leader: Søren Krogh Jensen
Responsible partners: AU and NIBIO, SLU, Trust'ing – Alf'ing, UCATT, IARTC

Overall summary of main results, discussion and conclusions of WP4

Field experiments in Sweden, Norway and Turkey showed that both leaf stripping and juice pressing increased protein content in one fraction and plant fibre content in the other fraction. The experiments demonstrated that juice pressing is more efficient in separating CP and plant fibre in different fractions than leaf stripping.

Overall, the juice fraction is a high protein, low fibre product due to the successful manner in which twin-screw pressing collects fibrous tissue in the pulp. Leaf stripping on the other hand is a high protein, high fibre product. This higher NDF concentration in the leaf fraction is likely due to the fibrous tissue in the petioles and upper stem that are also harvested when leaf-stripping.

Results also showed that the leaf stripper can be used in mixed stands of clover and grass, which is relevant for locations where clover is not grown as a monoculture. Potentially, farmers could use a leaf stripper machine opportunistically in fields where there is a high proportion of clover.

All the fractions derived from field stripping and juicing have a high moisture content that makes their preservation difficult. Direct silage without additives led to a highly acetic fermentation with more protein degradation. Various methods were tested to improve the ensiling process. Adding sugars or dry feeds (sugar beet pulp or barley meal) markedly improved the silage fermentative profile and reduced protein degradation. These results are useful for farmers planning to ensile the products from leaf stripping or juice pressing.

Report on the results obtained (A), and fulfilment of objectives (B) comparing to the original project proposal

A- results obtained and structured in relation to the user groups they are relevant for:
The aims of this work package were to 1) assess effects of forage legume species, number of harvest and fractionation method on yields and chemical composition and in vitro digestibility of the fraction; 2) test a leaf stripper harvester in mixed stands of forage legumes and grass; and 3) compare different preservation methods for protein-rich and fibre-rich fractions.

A4.1 Fractionation equipment was purchased (screw presses) or supplied (leaf stripping machines) by Trust'ing – Alf'ing. The electric PremAlfa Mini was developed specifically for the project needs.

A4.2-4. Field experiments were established in Northern Sweden, mid-Norway, Western France and Western Turkey in 2018. The forage legumes in experiments in Sweden and Norway were lucerne, red clover (2 varieties) and alsike clover. The field in Turkey had two varieties of lucerne. The field in France was abandoned due to drought. The fields were harvested 3 times in Sweden, 4 times in Norway and 7 times in Turkey in 2019. In 2020, the field in Norway was harvested 3 times. We prepared multiple fractions (Figures 2 and 3): the whole plant biomass, a leaf stripper fraction (mainly leaves), a residual leaf stripper fraction (mainly stems), a screw press fraction (juice), a residual screw press fraction (pulp), a pulp fraction, a leaf stripper juice fraction and a leaf stripper pulp fraction (Figure 2). However, due to the delay of the PremAlfa Mini leaf stripper the complete set of fractions was only produced from the third harvest in Sweden in 2019 and in Norway in 2020.

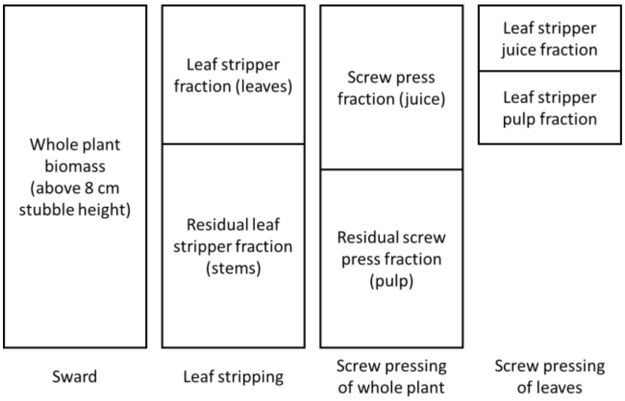


Figure 2. Fractions of whole plant biomass obtained by leaf stripping, screw pressing or a combination of both. The leaves and stems fractions contain also other plant parts.

Preliminary results of the first harvest in Turkey showed differences between the two lucerne varieties in yields and protein content (Erdal et al., 2020 https://orgprints.org/id/eprint/43271/). The leaf and the juice fractions had higher protein content than the whole plant. Preparing juice from leaves gave an additional increase in protein content.

Due to the issues that arose with acquiring the leaf stripper and the survival of experimental plots in Sweden following an icy winter, data collected during the 2020 harvest season in Norway provided the most complete dataset with which to compare the two fractionation methods. The following are initial quality and yield results of red clover harvested in Norway 2020 (Table 1). Both fractionation methods produced higher DM yields of the fibre fraction than the protein fraction. Juicing resulted in a higher DM yield of the protein fraction when compared to leaf-stripping. The average CP concentration of the protein fraction was comparable between juicing and leaf stripping, meaning that both were able to allocate similar amounts of CP to the protein fraction. For both methods, the protein fraction had a higher CP concentration than the fibre fraction. On average, juice had a much lower NDF concentration than the leaves, showing that juicing is more successful in distributing NDF to the fibre fraction than leaf stripping (Micke et al., in preparation, A4. 2-4). Overall, the juice fraction is a high protein, low fibre product due to the successful manner in which twin-screw pressing collects fibrous tissue in the pulp. Leaf stripping on the other hand is a high protein, high fibre product. This higher NDF concentration in the leaf fraction is likely due to the fibrous tissue in the petioles and upper stem that are also harvested when leaf-stripping.

Table 1: Means of yield and quality data for the four fractions produced with leaf stripper or screw press in Norway 2020

Variable	Juice	Leaf	Pulp	Stem
DM Yield (kg DM/ha)	526.1	354.2	670.1	826.4
CP (% DM)	22.9	22.1	15.6	16.0
NDF (% DM)	2.1	31.8	52.8	37.3

The following are some initial comparisons of the quality and yield of red clover between the two sites. Overall DM yields were higher in Sweden than in Norway. At both sites, juicing produced higher DM yield of the protein product than leaf stripping. In Norway, the CP concentration of the juice and leaves were comparable, while in Sweden CP concentration of the leaves was higher than the juice. In both countries, the NDF concentration was higher in the leaves than the juice (Micke et al., in preparation, A4. 2-4).

As in vitro digestibility analysis was not run on the juice fraction, digestibility of the juice and leaves cannot be compared. For the species harvested in Sweden (3rd cut 2019), the leaves from both lucerne varieties show higher in vitro total DM digestibility (IVTDMD) than the red clover varieties. For the fibre-rich fraction, the pulp had a higher IVTDMD than the stems. For the Norway 2020 dataset, there was no effect of variety on the digestibility of the leaves. For the fibre fraction, the pulp had a higher IVTDMD than the stems for all varieties (Chagas et al., in preparation, A4.2-4).

Preservation experiments. The protein-rich fractions were characterised by low dry matter content, but adding molasses, beet pulp or barley meal led to fermentation characteristics in the preserved products (A4.5-6 Bani et al., in preparation; A4.5-6 Bani et al., in preparation). Pulp fractions had a dry matter content suitable for fermentation, but sugar content was low. Adding molasses improved the fermentation characteristics of the pulp silages.

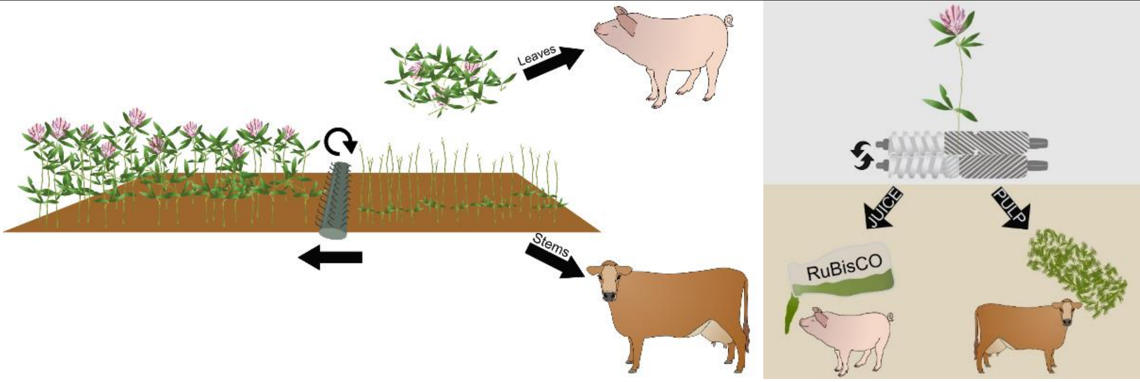


Figure 3. A leaf stripper (left) and a twin screw press (right) can be used to fractionate forage legumes into protein-rich feeds suitable for monogastrics and fibre-rich feeds suitable for ruminants (Illustrations: Brooke Micke). Leaves and juice have a high content of RuBisCO-protein and stems and pulp have a higher content of plant fibres compared to the whole plant.

Samples of different fractions from NIBIO, SLU and UCATT were analysed in the same lab (UCATT) for CP, ash, aNDFom and in vitro digestibility as well as their NIR spectra, for a total of 720 samples (vs the 100 planned in the project). Whole crop red clover had higher content of CP, but similar content of fibre than lucerne. Dry matter and fibre, in particular, were more digestible in red clover than in lucerne, though total fermentability measured as asymptotic in vitro gas production was similar for both forages. Differences between pulps and stems with regard to CP and fibre contents were not consistent, but pulps had a higher in vitro digestibility and rate of digestion. These differences in digestibility and digestion rate were more pronounced in lucerne (Bani et al., in preparation, A4.2-49) and less in red clover. Marked differences in chemical composition and digestibility were noted between countries, particularly between northern locations, Sweden and Norway, and Italy. The lucerne whole plant as well as its fibre-rich co-products contained more fibre and were less digestible in Italy compared to the other locations highlighting the influence of the cultivation environment of the forages feed values.

All the fractions derived from field stripping and juicing have a high moisture content that makes their preservation difficult. Drying requires high energy consumption and costs and, though ensiling would be the best preservation option, the low dry matter and chemical composition makes it difficult to achieve good preservation by direct ensiling. All the fractions, except juice, were ensiled without or with the addition of different additives, in particular microbial inoculants, sugars rich feeds and feeds commonly used in ruminants and monogastric nutrition. The experiments confirmed the results obtained with silages obtained at AU, i.e. that direct ensiling leads to a highly acetic fermentation and protein degradation, but adding sugars or dry feeds (sugar beet pulp or barley meal) markedly improves the silage fermentative profile and reduced protein degradation (Bani et al., in preparation, A4.5-6). Even juice, when mixed with beet pulps or barley meal appeared to be suitable for ensiling (Bani et al., in preparation, A4.5-6). However, further research is needed to better verify the true values of these preserved silages when fed to animals, both ruminants and monogastrics.

A4.7. A plot-scale leaf stripper was tested in mixed red clover-grass stands in Northern Sweden (Parsons et al., in preparation). The PremAlfa Mini leaf stripper worked well in mixed stands, removing on average a third of the available forage biomass, primarily in the form of clover leaves and soft stems. The forage fraction had a significantly higher CP concentration (+34%), slightly higher organic matter digestibility (+3.8%) and lower neutral detergent fibre (NDF) concentration (-27%) than the pre-harvest mixed sward.

B- fulfilment of objectives:

The project generated new knowledge about fractionation of forage legumes through leaf stripping, screw pressing and a combination of both methods. A direct comparison of the methods has to our knowledge not been done previously. Data from pure stands of forage legumes grown in regions where the methods have not been applied earlier add to the body of knowledge. We gained new knowledge from using a leaf stripper in mixed stands, and showed that the leaf stripper could be used in mixtures of clover and grass, and that it increased CP in the leaf stripper fraction.

Different preservation methods for protein-rich and fibre-rich fractions were tested, and practical suggestions for successfully ensiling these fractions were proposed in scientific and dissemination literature.

WP5	*Feed evaluation and animal feeding*

WP leader: Paolo Bani
Responsible partners: UCATT and INRAE, Trust'ing – Alf'ing, AU

Overall summary of main results, discussion and conclusions of WP5

The aim of this work package was to evaluate the nutritional value of protein-rich feed products (based on WP3 and WP4) in monogastrics and fibre-rich feeds in ruminants using in vitro and in vivo techniques, produce practical guidelines for animal feeding and NIR calibration for their fast and cheap evaluation. All the goals were achieved. The leaves and juice were nutritionally evaluated in pigs at INRA, stems and pulp on lambs at UCATT, all deriving from the same lucerne and red clover crops cultivated and processed at AU. Leaves and protein pastes from juice were tested at INRA to determine the total tract digestibility (TTD) of dietary nutrients and the standardized ileal digestibility (SID). TTD of energy was higher in leaves than in protein pastes and lower in lucerne vs. red clover. The SID of total essential AA of protein from juice was higher in lucerne than in red clover (87.2 vs. 79.2%). Green protein concentrates extracted from lucerne and red clover have great potential as a protein source for pigs. An experiment on finishing pigs suggests that silage made from lucerne leaves can be used up to an inclusion level of 10% and silage made from red clover leaves up to 20% in pig feed without any detrimental effect of growth performance. In two in vivo experiments with lambs, we used inclusion of 50% stems or pulp in the total DM intake. Growth performances, rumen fermentations, metabolic conditions and fatty acid composition were monitored, together with in vivo digestibility measurement. When part of balanced diets, stems and pulp obtained from lucerne and red clover have the potential to be used for ruminants feeding up to 50% of total dietary dry matter. Chemical composition and in vitro digestibility was measured on more than 600 samples, differentiating between species and cultivation areas, with southern grown crops being less digestible than northern ones. The analysis of these samples allowed to produce a wide set of NIR calibrations for a fast and cheap evaluation of their nutritional traits, most of which are already valuable for their practical use and freely available on request. Some changes were made from the original project but all the objectives originally envisaged have been achieved.

Report on the results obtained (A), and fulfilment of objectives (B) comparing to the original project proposal

A- results obtained and structured in relation to the user groups they are relevant for:

The aim of this work package was to evaluate the nutritional value of protein-rich feed products (based on WP3 and WP4) in monogastrics and fibre-rich feeds in ruminants using in vitro and in vivo techniques.

According to the plan, two forage legumes, lucerne and red clover, were cultivated by AU. First cut lucerne harvested in May 2019 and second cut red clover harvested in August 2019 were processed and fractionated into juice and pulp, leaves and stems. To maintain their original nutritional quality for a long duration and to ensure that they remain easily available for feeding pigs, leaves were preserved by ensiling them with 20% of organic barley. Protein contained in juices were concentrated by the above-mentioned technology in order to obtain pastes (PP) with 28 to 30% dry matter and with 54 to 56% CP contents. The aim of the work was to determine the nutritional value of silages (S) from the whole plant of lucerne (L) and red clover (R) and PP obtained L and R leaves from lucerne (L) and red clover (R).

In a first experiment, 30 pigs were used in a factorial design to determine the TTD of dietary nutrients in five dietary treatments LS, RS, LPP, and RPP. The control group was fed a control diet (C1). The LS and RS groups were fed a 78%:22% mixture (on a DM basis) of the C1 diet and LS or RS. The LPP and the RPP groups were fed an 81%:19% mixture (on a DM basis) of the C1 diet and LPP or RPP. In the second experiment, five pigs were used in a 5 × 5 Latin square design to evaluate the standardized ileal digestibility (SID) of amino acids (AA) in the four legume products. The control diet (C2) was formulated with casein as the sole protein source. The LS and RS groups were fed an 85%:15% mixture (on a DM basis) of the C2 diet and LS or RS. The LPP and RPP groups were fed an 80%:20% mixture (on a DM basis) of the C2 diet and LPP or RPP.

Regardless of the plant species, silages contained less AA and more fibre than protein pastes. While the fresh forages contained the same percentage of protein N in total N (63.6%), lucerne lost more protein N during ensiling than red clover (-75.5 vs -33.8%). The calculated TTD coefficient of energy was higher in silages than in protein pastes and lower in R than in L products (72.8, 71.5, 67.7, and 61.3 for LS, RS, LPP and RPP, respectively). The SID of total essential AA was higher in LPP than in RPP (87.2 vs. 79.2%). This effect of plant species on protein digestibility was already reported in the literature. Higher CF content and percentage of N associated with the NDF fraction could partly explain the lower N SID in RPP. The amounts of SID lysine provided by LPP and RPP were higher than or similar to the mean value reported for soya bean meal (31.4 and 27.3 vs. 28.4 g/kg DM, respectively). Similar results were found for the other essential and non-essential AA, which suggests that green protein concentrates extracted from lucerne and red clover have great potential as a protein source for pigs. The SID of total essential AA was lower in LS than in RS (33.2% vs. 56.8%). The lower SID values in silages were explained by the protein degradation during the ensiling process and a high proportion of AA linked to the NDF fraction. That suggests that legume silages (especially lucerne silage) have to be considered as an energy source rather than a protein source.

In a second step, we evaluated the effects of two levels of dietary inclusion of lucerne leaves (LLS) and red clover (RLS) on growth performance and carcass traits of finishing pigs. A total of 60 growing pigs were divided into 5 treatments designed to provide the same daily amount of metabolizable energy (EM, i.e., 37.4 MJ/d) for a fixed daily intake of DM (i.e., 2.6 kg/d). Animals from treatment 1 (T1) were fed a standard diet formulated from cereals (maize, barley, wheat), wheat bran and soybean meal (12.5 MJ ME / kg and 0.45 g SID lysine / MJ IN). Animals from T2 and T3 were fed with a mixture of 90% of the standard diet and 10% ensiled LLS or RLS. Animals from T4 and T5 received a mixture of 80% of the standard diet and 20% of LLS or RLS. Treatments influenced ($P < 0.05$) both average day gain (ADG) and feed conversion rate (FCR), whereas pigs from the T4 group showed a lower performance when compared to T3 and T5 (783 vs. 885 g/ d; 3.23 vs. 2.89 kg/ kg, respectively for ADG and FCR on average) and final body weight was also significant ($P = 0.023$), where pigs from T4 showed a lower BW at end when compared to the other treatments (109 vs. 111 kg). In conclusion, our results show that lucerne and red clover leaves silages can partially replace traditional cereals without reducing performance. In practice, due to its low protein value, it is not recommended to use more than 10% lucerne silage in pig feed. However, finishing pigs can be fed with red clover silage up to 20% without any detrimental effect of growth performance.

Stems and pulp were ensiled in plastic barrels and sent to UCATT in November 2019 where they were used in two in vivo trials carried out on lambs. Two in vivo experiments were carried out on 18 lambs each. Stems and pulp from lucerne and red clover were used to formulate 4 diets, where these products represented 50% of the total dry matter, to highlight their influence on animals' performance. Growing performances, rumen fermentations, metabolic conditions and fatty acid composition were monitored, together with in vivo digestibility measurement. All the diets were well accepted by the animals and resulted in good performance. When part of balanced diets, stems and pulp silages result in similar growing and health results. Average dry matter intake was 3.90 and 3.55% of live weight for lucerne and red clover-based diets, respectively whereas average daily gain was 566 and 475 g/d, respectively, without significant differences between diets based on stems or pulp. Compared to pulp, stems always contained less protein but not more fibre in clover. Accordingly, diet with pulps had higher digestibility vs stems-based diet for lucerne but not for red clover. Fat composition was more valuable with regard to human nutrition for lambs fed pulp silages compared to pulp silages, because of a higher content of many unsaturated fatty acids and in particular of omega-3 fatty acids and of CLA precursor vaccenic acid. Stems and pulp obtained from lucerne and lucerne seems to be suitable for lambs, and likely for other ruminants, feeding also in relevant proportions of their diets, though further experiments are needed to define more robust practical indications.

B- fulfilment of objectives:

We fulfilled the objective to evaluate the nutritional value of protein-rich feed products in monogastrics and fibre-rich feeds in ruminants using in vitro and in vivo techniques.

All experimental feeds were produced at AU to ensure that feeds used in experiments with pigs at INRAE were related to the feeds used in experimental with lambs in Italy. The digestibility of the protein-rich and the fibre-rich fractions were measured, and nutritional strategies and recommendations described. Samples of forage legumes and their fractions from the field experiments (WP4) were analysed chemically and digestibility in fibre-rich fractions was assessed with in vitro methods. In vitro methods for assessment of digestibility in the protein-rich fractions did not give reliable results. The NIRS calibrations were not performed in pig feed, but were compensated by an additional experiment (not initially planned in the project) aiming to evaluate the practical conditions (rate of incorporation, formulation of the concentrate, modalities of feed distribution) of the use of leaf forage silages in pig feeding. Preservation techniques were investigated for both fibre-rich (stems and pulp) and protein rich (leaves and juice) feeds and already applicable guidelines were produced. Finally, we developed NIRS-calibrations for several of the fibre-rich fractions.

WP6	*Sustainability assessment of local food systems and farmer attitudes towards self-sufficiency*

WP leader: Steffen Adler
Responsible partners: NIBIO, Ruralis and all partners

Overall summary of main results, discussion and conclusions of WP6

The overall idea of implementing a method for forage legume fractionation is to develop animal-based food systems that are based on local feeds, with the aim to make food production in organic farming more sustainable. In the assessment of sustainability, social, economic and environmental aspects must be considered. Leaf stripping and juice pressing are generally not commercially implemented methods and therefore it was not surprising that farmers' and stakeholders' perceptions were influenced by lack of information. Stakeholders had a positive view on self-sufficiency in feed, but for farmers producing for export this was less important. Low level of commercial implementation also limited possibilities for data collection and limited assessment of economic and environmental aspects. Therefore, scenarios and model farms were developed. The results of this work showed that cooperation between a dairy farm and a pig farm on leaf stripping or juice pressing will require minor changes in land use and the level of self-sufficiency will only increase on the pig farm. If we assume that ensiled leaves and protein concentrate from forage legumes can replace 10% of pig feed both methods have similar benefits, however, Renaudeau et al. (submitted) found that leaf silages are more an energy than a protein source for pigs. On the other hand, investment costs are much higher when applying a press screw than a leaf stripper. An estimation of Net Present Value showed that both models can be feasible, but the profitability depends on the scale of production and the specific assumptions. More information and further assessments are necessary for a more reliable assessment of sustainability in local food systems based on fractionation of forage legumes in organic animal production.

Report on the results obtained (A), and fulfilment of objectives (B) comparing to the original project proposal

A- results obtained and structured in relation to the user groups they are relevant for:

This part of the project aimed to gain more knowledge about farmers' and other stakeholders' attitudes towards self-sufficiency and views on local food systems, and to assess aspects of sustainability for scenarios of local food systems. We used Norway, Sweden, Denmark and Turkey as cases. Currently, neither leaf stripping nor juice pressing of forages are commercially implemented in Norway, Sweden or Turkey. In Denmark, implementation of juice pressing on commercial farms has recently started. As a starting point, we developed two concepts of local food systems that involve cooperation between farmers and feed industry or cooperation between farmers only (Figure 4).

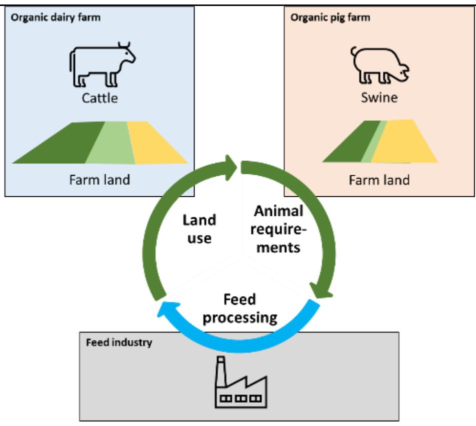


Figure 4. Concepts of a value chain based on local feed production through forage legume fractionation and cooperation between farmers and feed industry (green and blue arrows) or only between farmers (green arrows).

To gain information of farmers' and stakeholders' views on self-sufficiency in feed and what barriers and possibilities they envision for the implementation of the presented concepts we conducted in-depth interviews with farmers and focus group interviews with stakeholders. Norwegian and Danish farmers were interviewed about self-sufficiency, where Norwegian farmers were more positive than Danish farmers, especially the Norwegian pig farmers (Logstein et al., report manuscript). A possible explanation may be that Danish pig farmers were large scale and produced mainly for international markets whereas Norwegian pig farmers were small scale, more flexible, with a closer relation to consumers that allowed farmers to meet their preferences.

Stakeholders in Sweden and Norway were interviewed about attitudes towards self-sufficiency in feed and were in general positive. In both countries, they discussed how self-sufficiency could relate to various spatial scales, such as farm, regional, national and Nordic level. They proposed that self-sufficiency is important of different reasons, such as food security, nutrition balance, moral responsibility, employment in rural areas, and consumers acceptance and branding. As challenge mentioned (among others) was goal conflicts between climate emission and resource utilisation, shortage of available land, farm economy, and logistical and structural barriers.

When it comes to attitudes to the two models for cooperation, the main challenges mentioned by the farmers in Norway and Denmark, that were interviewed, were assumed low feed value of the pulp fraction for dairy cows, laborious production process, lack of available land, and increased feed costs for pig farmers. Danish farmers stressed the need of fair and sustainable agreements in a cooperation between farmers. When Danish farmers preferred concept 2 that involves the feed industry for safer feed production, most Norwegian farmers preferred concept 1. Farmers also suggested other models for cooperation such as cooperation with crop farmers, establishment of a link to biogas plants and establishment of co-operatives to produce local protein.

Challenges related to feed preservation, shortage of land, high investment costs, and logistics were mentioned by stakeholders in all countries interviewed, i.e., Norway, Denmark and Turkey. On the other hand, local value chains could increase employment in rural areas. Involving the feed industry can increase product value through improved quality and homogeneity, but it may also increase feed costs. In Turkey, stakeholders mentioned lack of trust between actors as a barrier, and both Norwegian and Turkish stakeholders mentioned a challenge connected to national subsidies of feed.

We calculated details of land use, processing and animal requirements in two models of local value chains. These calculations were done for conditions in mid-Norway. An organic dairy farm with 40 dairy cows and 50 ha of land and an organic pig farm with 30 sows and 820 fattening pigs per year, and 30 ha of land were used as the baseline to calculate the models. The land use included meadows with clover grass, pastures and land for cereal crops. Crop yields were assumed to be the same on both farms. We kept crop yields and animal performance constant when developing the models, but adjusted land use and exchange between farms and delivery and purchases from the feed industry to estimate effects on self-sufficiency. The baseline farms were self-sufficient with forages with the amendment that the dairy farm bought the surplus forage from the pig farm.

Model 1 was an implementation of leaf stripping on both farms and the feed industry was not involved. We assumed that 2nd and 3rd harvest of clover grass was used for leaf stripping. Furthermore, we assumed that ensiled clover leaves could substitute 10% of the purchased pig feed. Land use on both farms was adjusted to keep the dairy farm self-sufficient in forage feeds.

Model 2 was an implementation of juice pressing on both farms and here the feed industry was involved in processing the press juice to a component used in pig feed. We assumed that 2nd and 3rd harvest of clover grass was used for juice pressing. Furthermore, we assumed that protein concentrate from the juice could substitute 10% of the purchased pig feed. Land use on both farms was adjusted to keep the dairy farm self-sufficient in forage feeds.

Self-sufficiency in feed protein, was 80% at baseline for the dairy farm and did not change in Model 1 and 2. Here we treated feed produced on the cooperating farm as own feed. For the pig farm self-sufficiency increased from 24% to 74% in both models. To achieve this, forage area had to increase on the pig farm in both models. This reduced the level of self-sufficiency in cereals on the pig farm in both models slightly.

We assumed that silages of whole plant forage, pulp and stems on one hand and juice protein concentrate and leaf silage on the other hand had similar feed values. However, results from WP4 (Renaudeau et al., submitted; Bani et al., manuscript) indicate that whole plant silage and pulp silage have higher feed value in ruminants than stem silage, and whereas protein concentrate can be considered as a valuable protein feed for pigs, leaf silage can be considered as an energy feed for pigs. However, with the assumptions used in our calculations, cooperation in fractionation of forages between a dairy farm and a pig farm has the potential to lift the level of self-sufficiency of the pig farm to the level of that of the dairy farm, which was already high in the baseline. Required changes in land use are rather small, but processing, transportation and investment costs are barriers that need further investigation and innovation.

We investigated if there were economic incentives for farmers to produce/procure local feeds based on forage fractionation. We assessed the economic profitability of Model 1 and Model 2 for feed procurement in dairy and pig organic farms by performing a cost-benefit analysis. Net Present Value (NPV) was used as an evaluation criterion, which represents the sum of a projects net benefits discounted over the lifetime of the investment/project, or – if net benefits are assumed to be constant over the investment lifetime – on the net benefits of a single year. While our results suggest that both models can be feasible (NPV > 0), the profitability depends on the scale of production and the specific assumptions (e.g., factor prices, which reflect the prices of production factors) that we made.

B- fulfilment of objectives:
We fulfilled the objectives to develop concepts of local food systems based on forage legume fractionation and to explore farmers' attitudes towards self-sufficiency and cooperation in local food systems. The objective to assess sustainability was at least partly fulfilled. We assessed two concepts and two models of local food systems. Collection of data about regional differences and dissemination with a participatory approach was more challenging and thus the assessment has less focus on regional differences than what we had planned. Covid-restrictions made data collection more difficult and dissemination with a participatory approach almost impossible. Due to these difficulties, we found the data not suitable for a peer-reviewed article, instead we wrote one report manuscript to cover all topics in WP6.

3.2 Deliverables and milestones status

Deliverable No.	Deliverable name	Link to the document[2)]	Planned delivery month[1)]	Actual delivery month[1)]	Reasons for changes/delay and explanation of consequences in case of delay, if any

D1.1	Experimental procedures	Internal	3	10	Delayed, but the documents were ready before start of the experiments
D2.1-4	Stakeholder group meeting	Internal meetings	31	36	In most participating countries 2 meetings were carried out. A third round was difficult to organise due to Covid restrictions. Data collected in the focus group interviews were sufficient for D6.3.
D2.5	National field days	Video from field day in Norway (editing in progress) and summery from field day in Turkey (in progress)	32	42	Field days were carried out in Norway and Turkey. In the other countries it was not possible to arrange field days due to Covid restrictions.
D2.6	Written information for dissemination	Leaflet: https://www.nibio.no/en/projects/prorefine?locationfilter=true https://projects.au.dk/fileadmin/user_upload/prorefine_leaflet_web.pdf Poster: https://orgprints.org/id/eprint/36989/ https://orgprints.org/id/eprint/36855/ https://orgprints.org/id/eprint/36989/ Newsletter: https://orgprints.org/id/eprint/43586/	33	41	We have prepared 2 leaflets, 2 posters, 3 newsletters, 1 practice abstract and 1 contribution in a professional journal. 2 more practice abstracts and 1 working paper are in final editing progress.

		https://orgprints.org/id/eprint/43584/ https://orgprints.org/id/eprint/43585/ Practice abstract: https://orgprints.org/id/eprint/43202/ Professional journal: Vallbrev Other: https://orgprints.org/id/eprint/36856/			
D3.1	Models for forage legume production		30		In progress (A3.1, see Chapter 4.5)
D4.1-4	Field experiments on fractionation	Conference contribution: https://orgprints.org/id/eprint/43504/ https://orgprints.org/id/eprint/43090/ https://orgprints.org/id/eprint/42311/ https://orgprints.org/id/eprint/43271/	34	41	Field experiments were carried out in Sweden, Norway, Italy and Turkey. Harvesting and processing was repeated in Sweden and Norway in 2020, because the leaf stripper arrived late in 2019. The field experiment in France was not successful due to unfavourable weather conditions. The field experiment planned in Denmark was cancelled to free resources to produce experimental feeds for the feeding experiments. 3 conference contributions. Several publications in progress and 2-3 articles will be part of Brooke Micke's doctoral thesis (A4.2-4: 3 manuscripts, A5.3: 1 manuscript and 1 manuscript for internal use see Chapter 4.5).
D4.5-6	Preservation experiments		34	42	In progress (A4.5-6: 2 manuscripts, see Chapter 4.5).
D4.7	Leaf stripping in mixed stands	Videos:	35		In progress (A4.7: 1 manuscript, see Chapter 4.5).

		https://www.nibio.no/en/projects/prorefine/dissemination?locationfilter=true			
D5.1-3	Nutritional value and digestibility–protein-rich feeds	https://orgprints.org/id/eprint/43111/ https://orgprints.org/id/eprint/43112/	34		1 article submitted and in revision process (A5.4-5, see Chapter 4.5). 2 conference contributions.
D5.1-4	Nutritional value and digestibility–fibre-rich feeds		35		In progress (A5.4-5: 2 manuscripts, see Chapter 4.5).
D5.5	Nutritional strategies and recommendations		35		In progress. 1 working paper in final editing phase and will be submitted to Organic Farm Knowledge website.
D6.1-2	Self-sufficiency interviews and questionnaires		34		In progress (A6.1-3: 1 manuscript for professional report, see Chapter 4.5).
D6.3	Sustainability assessment	https://orgprints.org/id/eprint/36857/	35		Will be part of the report (D6.1-2). 1 presentation of the project with focus on sustainability.

1) Measured in months from the project start date (month 1)
2) E.g. documents as orgprints.org/33121 or other types of deliverable (e.g. APPs or devices)

Milestone No.	Milestone name	Planned delivery month[3)]	Actual delivery month[3)]	Reasons for changes/delay and explanation of consequences, if any.
M1.1	Project meeting, kick-off	3	4	Completed
M1.2	Project meeting, mid-term	15	14	Completed
M1.3	Project meeting, final	33	January 2022	Face-to-face meetings could not be arranged due to Covid restrictions. We organised regular online project meetings during the course of the second half of the project. We collected recorded presentations and videos on the project webpage to make project results available. Further presentations will follow after articles have been published.

				https://www.nibio.no/en/projects/profine?locationfilter=true
M2.1	National stakeholder groups	3	13	The Danish group was established later and meeting 1 and 2 were combined.
M2.2	National stakeholder group meetings I	6	13	In some countries it was more challenging to find relevant stakeholders that were willing to participate.
M2.3	National stakeholder group meetings II	19	24	Some delays due to Covid restrictions.
M2.4	National stakeholder group meetings III	30	-	Not carried out due to Covid restrictions. We collected sufficient data during round 1 and 2 for M6.3.
M3.1	Models for forage legume production	21	January 2022	Insufficient data was collected in 2019 due to late arrival of equipment, and more data was collected in 2020 and 2021.
M4.1	Leaf stripper harvester / juice squeezer	9 / 3	18	The development and production of the MRFE was delayed. It has been used successfully at the third harvesting in Sweden and in additional harvesting in 2020.
M4.2	National field experiments	9	9	Fields in Sweden, Norway and Turkey were established by month 9. The fields were also harvested in 2020. No fields were established in Denmark and Italy, where we prioritised extended feeding production experiments. The field experiment in France was abandoned due to drought. In Italy, fields of lucerne have been utilised for sample production and data collection.
M4.3	Field experiments, harvesting and fractionation	21	29	Additional harvest in Sweden and Norway in 2020.
M4.4	Field experiments, analyses	24	42	Delayed because of additional samples from 2020. Data part of PhD work.
M4.5	Preservation experiment Italy	9	42	Delay due to unfavourable weather conditions in 2018.
M4.6	Preservation experiment Norway	18	42	Activity was carried out by UNICATT.

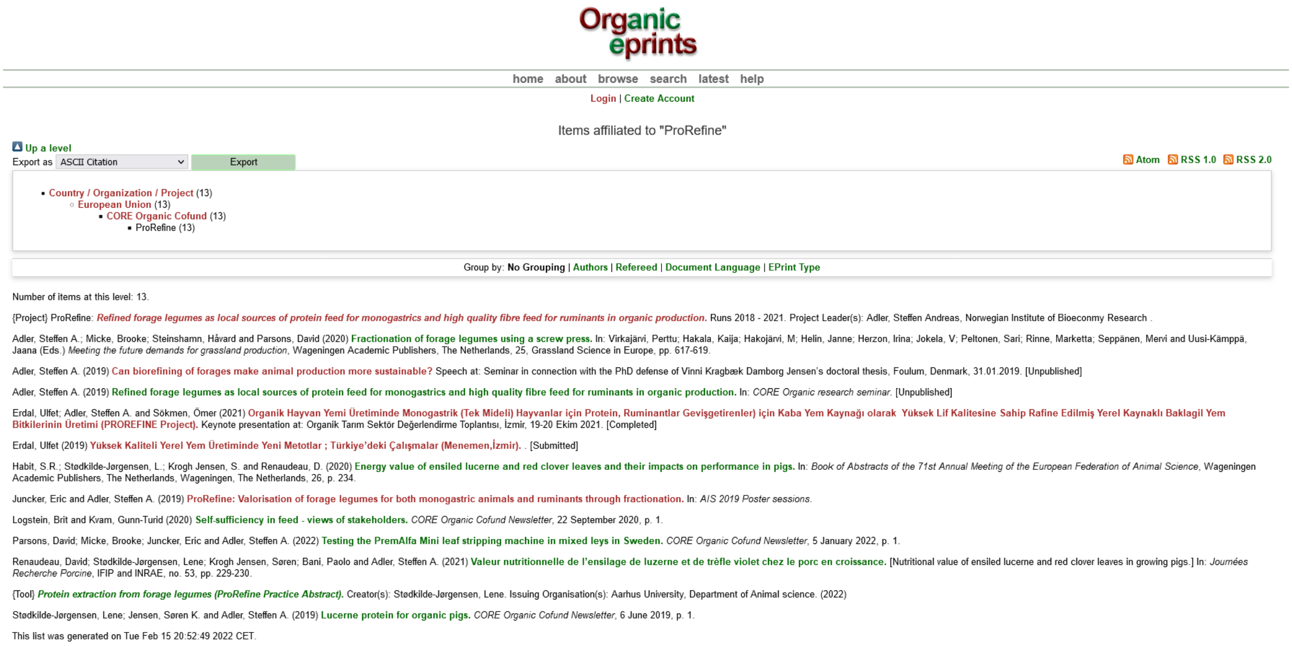
M4.7	Leaf stripping in mixed stands	18	January 2022	The activity began in 2020, but more data was needed so it continued in 2021.
M5.1	Experimental feeds for in vivo experiments	21	19	All experimental feeds were produced in Denmark and shipped to France and Italy.
M5.2	Analysis of samples from WP4	22	28	Additional samples from 2020 were analysed.
M5.3	Calibration of near infrared spectroscopy spectra	23	January 2022	Additional samples from 2020 were included.
M5.4	In vivo digestibility in pigs	24	42	An additional feeding experiment was carried out aiming to evaluate the practical conditions of the use of leaf forage silages in pig feeding.
M5.5	In vivo digestibility in dairy cows	24	January 2022	We used lambs instead of cows. Delay due to Covid restrictions.
M6.1	Concepts and scenarios of local food systems	9	14	Delay had no negative effect on project progress.
M6.2	Farmer interviews in Norway and Denmark	18	22	Delay had no negative effect on project progress.
M6.3	Sustainability assessment	27	January 2022	Data collection was more challenging than expected due to Covid restrictions and delayed project activities.

3) Measured in months from the project start date (month 1)

4. Publications and dissemination activities

4.1 List extracted from Organic Eprints

By 15 February 2022 13 items were affiliated to ProRefine in Organic Eprints (https://orgprints.org/view/projects/ProRefine.html). Two items lack affiliation to ProRefine and are listed below the screenshot.



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Items affiliated to "ProRefine"

Up a level

Export as ASCII Citation Export

Atom RSS 1.0 RSS 2.0

- Country / Organization / Project (13)
  - European Union (13)
    - CORE Organic Cofund (13)
      - ProRefine (13)

Group by: **No Grouping** | Authors | Refereed | Document Language | EPrint Type

Number of items at this level: 13.

{Project} ProRefine: ***Refined forage legumes as local sources of protein feed for monogastrics and high quality fibre feed for ruminants in organic production.*** Runs 2018 - 2021. Project Leader(s): Adler, Steffen Andreas, Norwegian Institute of Bioeconmy Research .

Adler, Steffen A.; Micke, Brooke; Steinshamn, Håvard and Parsons, David (2020) **Fractionation of forage legumes using a screw press.** In: Virkajärvi, Perttu; Hakala, Kaija; Hakojärvi, M; Helin, Janne; Herzon, Irina; Jokela, V; Peltonen, Sari; Rinne, Marketta; Seppänen, Mervi and Uusi-Kämppä, Jaana (Eds.) *Meeting the future demands for grassland production*, Wageningen Academic Publishers, The Netherlands, 25, Grassland Science in Europe, pp. 617-619.

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Stødkilde-Jørgensen, Lene; Jensen, Søren K. and Adler, Steffen A. (2019) **Lucerne protein for organic pigs.** *CORE Organic Cofund Newsletter*, 6 June 2019, p. 1.

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The following contributions lack a link to ProRefine in Organic Eprints:

Ulfet, Erdal and Adler, Steffen Andreas (2021) Protein From Fractionated Forage Legumes As Feed For Monogastric Animals. Paper at: Organic World Congress 2021, Science Forum: 6th ISOFAR Conference co-organised with INRA, FiBL, Agroecology Europe, TP Organics and ITAB, Rennes, France, 8 - 10 September, 2021. https://orgprints.org/id/eprint/42311/

The presentation of the above paper:

Erdal, Ulfet; Adler, Steffen A. and SUTAY, Serap (2021) PROTEIN FROM FRACTIONATED FORAGE LEGUMES AS FEED MONOGASTRIC ANIMALS (PROREFINE PROJECT). Keynote presentation at: Organic World Congress, Rennes, France, 6-10 September 2021. https://orgprints.org/id/eprint/43271/

4.2 *Stakeholders oriented articles in the CORE Organic newsletter*

Newsletter relevant for farmers, advisory service, farming machinery industry

Testing the PremAlfa Mini leaf stripping machine in mixed leys in Sweden (Parsons, Micke, Juncker & Adler, 2022)
https://projects.au.dk/coreorganiccofund/news-and-events/show/artikel/testing-the-premalfa-mini-leaf-stripping-machine-in-mixed-leys-in-sweden/

Lucerne protein for organic pigs (Stødkilde-Jørgensen, Jensen & Adler, 2019)
https://projects.au.dk/coreorganiccofund/news-and-events/show/artikel/lucerne-protein-for-organic-pigs/

Newsletter relevant for all stakeholders in the value chain of locally produced animal based products

Self-sufficiency in feed - views of stakeholders (Logstein & Kvam, 2020)
https://projects.au.dk/coreorganiccofund/news-and-events/show/artikel/self-sufficiency-in-feed-views-of-stakeholders/

4.3 *Practice abstracts*

Protein extraction from forage legumes (Stødkilde-Jørgensen, 2021).
https://organic-farmknowledge.org/tool/43202

Manuscripts of additional practice abstracts in progress

A4.5-6: Ensiling legume forage pulp (Bani, ready for submission to Organic Farm Knowledge)
A4.5-6: Ensiling legume forage stems (Bani, ready for submission to Organic Farm Knowledge)

4.4 Other dissemination activities and material

Working papers

A5.5: Legume forage stems and pulp in animal nutrition (Bani, ready for submission to Organic Farm Knowledge)

A5.5: Ensiling legume forages for feeding pigs (Renaudeau, in progress)

Video presentations:

We have prepared voice-over presentations of the project aims (A2: English) and leaf stripping in mixed stands (A4.7: English and Swedish). The videos are available from NIBIO's project page (https://www.nibio.no/en/projects/prorefine?locationfilter=true) and will be made available on SLU's project page (https://www.slu.se/en/departments/agricultural-research-northern-sweden/research/ongoing-research-projects/prorefine/).

Public Talks:

- ProRefine summary. Parsons. November 2020. Jordbruksverket organic agriculture workshop. (A2).
- Predicting forage quality. Parsons. January 2019. Aarhus University. (A3.1).
- Presentation of ProRefine. Adler. January 2019. Aarhus University. https://orgprints.org/id/eprint/36857/ (A2).
- ProRefine agronomy experiments. Micke. December 2021. Swedish subject committee on leys and forage. (A4.2-4).
- Protein from fractionated forage legumes as feed for monogastric animals. Presentation at: Organic World Congress 2021. Erdal. September 2021. https://orgprints.org/id/eprint/43271/ (A5.4-5).

Teaching material:

The project has also been presented during the following official courses at the Università Cattolica del Sacro Cuore of Piacenza

- Animal Husbandry (years 2019/2020 and 2020/2021). (A5).
- Organic Livestock Farming and Quality of Food of Animal Origin (2020/2021). (A5).
- Course for farmers and technicians "New tools for increasing the profitability of mountain agriculture and animal husbandry" (Nuovi orientamenti per l'incremento della redditività dell'agricoltura e zootecnia di montagna), Borgotaro (Parma), 1 – 23 July, 2021. (A5).

Other dissemination articles:

ProRefine – protein från vallbaljväxter. Parsons & Micke. Vallbrev (Newsletter of the Swedish Grassland Society). (A2). Vallbrev .

4.5 Future dissemination actions

Future dissemination activities are presented with activity number from project proposal, work title and a preliminary list of co-authors.

Manuscripts submitted to a journal and currently in peer review process:

A5.4-5: Nutritional values of forage-legume-based silages and protein concentrates for growing pigs (Renaudeau, Jensen, Ambye-Jensen, Adler, Bani & Stødkilde-Jørgensen, final review phase)

Manuscripts of articles intended for publication in peer reviewed journals:

A3.1: Models for predicting quality of bio-refined forages (Parsons, Micke and Adler, planned manuscript).

A4.2-4: Chemical and nutritional evaluation of alfalfa forage fractions obtained by green biorefining techniques (Bani, Jensen, Adler, Parsons, Renaudeau, Juncker & Stødkilde-Jørgensen, manuscript)
A4.2-4: Production and quality of clover and lucerne fractions produced by juicing and leaf stripping (Micke, Adler, Forkman & Parsons, manuscript)
A4.2-4: Nutrient value of forage legumes fractions and their viability for livestock in Nordic countries (Chagas, Micke, Parsons, Bani & Adler)
A4.5-6: Ensiling stems and pulp co-products after alfalfa fractionation or biorefinery (Bani, Jensen, Adler, Parsons, Renaudeau, Juncker & Stødkilde-Jørgensen, manuscript)
A4.5-6: Ensiling leaves and whole plant juice co-products after alfalfa fractionation or biorefinery (Bani, Jensen, Adler, Parsons, Renaudeau, Juncker & Stødkilde-Jørgensen, manuscript)
A4.7: Testing a leaf stripping machine in mixed leys of grass and clover (Parsons, Bergqvist, Micke & Adler, manuscript)
A5.3: Analytical and instrumental evaluation of errors for NIR miniaturized spectroscopy: the case study of forages (Giulia, Taiana, Boqué, Bani, Gachiuta & Giussani, manuscript)
A5.4-5: Effects of diets based on stems and pulp silages obtained from biorefining alfalfa or red clover whole plants on digestibility, growth performance, metabolic status and fat fatty acid composition in fattening male lambs (Bani, Jensen, Parsons, Renaudeau, Juncker & Adler, manuscript)
A5.4-5: Research note : Utilization of ensiled leaves from lucerne and red clover on growth performance of finishing pigs (Habit, Stødkilde-Jørgensen, Jensen, Ambye-Jensen, Adler, Silva, Bani & Renaudeau, manuscript)

Manuscripts intended for publication as professional reports:
A6.1-3: Green biorefinery – a way to improve sustainability in organic animal husbandry? (Logstein B., Rodriguez D.G.P., Adler S., Kvam G-T., Erdal Ü. Kudahl A.B., & Bernes G., manuscript)

Material not suitable for publication, but valuable for internal use:
A5.3: Developing NIRs calibrations for rapid and cheap evaluation of the chemical composition and nutritional value of feeds obtained by alfalfa and clover fractionation or biorefinery (Bani, Jensen, Parsons, Renaudeau, Juncker & Adler, manuscript, not peer reviewed)

Videos and voice over presentations, in progress
A2.5: Video recording of field day in Tingvoll
A4.2-4: Presentation of field experiments and their results

4.6 Specific questions regarding dissemination and publications

The webmaster of the CORE Organic Cofund project website has confirmed that the website is up-to-date. We intend to add video presentations when they are all completed. Video presentations will also be made available from NIBIO's and SLU's project websites.
https://projects.au.dk/coreorganiccofund/core-organic-cofund-projects/prorefine/

https://www.nibio.no/en/projects/prorefine?locationfilter=true

https://www.slu.se/en/departments/agricultural-research-northern-sweden/research/ongoing-research-projects/prorefine/

We see farmers, local entrepreneurs, various stakeholders in the feed and food industry, advisors in agriculture and animal husbandry, the scientific community, NGOs, GOs, and the general public as the main user groups of project results. Through stakeholder group meetings in each country, we created a platform to discuss project plans and results.

Leaf stripping and juice pressing are only exceptionally implemented in commercial farming. Interviews revealed that many dairy farmers are sceptical to selling forage protein from their farms. And the feed industry considers implementation of fractionation methods to be connected to a high level of uncertainty and risk.

Unfortunately, we were not able to carry out a third round of stakeholder group meetings and only two field days due to Covid restrictions. This limited our possibilities to disseminate project results directly to stakeholders. Instead, we prepared several voice-over presentations that are available at NIBIO's project website (https://www.nibio.no/en/projects/prorefine/dissemination?locationfilter=true).

5. Project impact

The proposed project was designed to produce new knowledge about production and nutritional values of feed for monogastrics and ruminants by fractionating and processing forage legumes in organic farming. A potential future impact of the project is increased availability of locally produced concentrated protein feeds which may increase the level of self-sufficiency and possibly viability of local food systems.

The project has gained new knowledge about how forage legume species, number of harvest and fractionation method affect yields and fraction composition. When these results are published (see list of manuscripts in chapter 4) farmers can with support from advisors implement this new knowledge in practical farming. The results will provide the feed industry with data to assess how fractions of forages legumes may be included in feed products. Promising results with leaf stripping in mixed stands of clover and grass expand the usability of the experimental harvester developed and tested in the project. However, fractionation of forage crops and their utilisation requires specific equipment. Additionally, the implementation of juice pressing will require adaptation in the entire value chain.

Demonstrations of equipment and processes were only partly possible to carry out, but this may be integrated in future field days. Improvements in preserving pulp, leaves and press juice have been achieved in the project and may even be applied to other feeds with either low dry matter or low sugar content. Results from feeding experiments with pigs and lambs will allow a more precise calculation of balanced diets for organically raised monogastrics and ruminants. This knowledge will help farmers and feed industry to maximise utilisation of local feeds. Implementation will most likely lead to new food products demanded by consumers supporting local food systems.

New knowledge about the economic, environmental and social sustainability of local food systems in the context of regional conditions strengthens local entrepreneurship and value creation in rural areas. The overall effect on general sustainability will be improved through adaptability to regional conditions because sustainability must be a goal at different levels. Farmers, feed industry, food industry and the society in general can utilise this knowledge to develop more sustainable food systems and diets.

New knowledge on farmer attitudes towards self-sufficiency, motivation to cooperate in new ways and ability to deal with risk management could be transferred to conventional agriculture. It increases the awareness of resource utilisation and recycling in agriculture and among consumers. Finally, the forage legume-based feeds may have effects on product quality and consumer preferences.

Further research and technology development is necessary to strengthen the impact of this project on the development of local food chains. In 2021, two spin-off projects started. In Turkey a project has been initiated by Ülfet Erdal to study the effects of lucerne grown in organic and conventional farming systems on soil properties, yield and CO_2 emissions (GDR/TAGEM, 2021-2025). In Norway, a project on locally sourced feed protein for organic pig production was funded (NØFF, 2021-2023, Landbruksdirektoratet). One of the project aims is to develop leaf stripping in mixed stands further. We expect more spin-off projects developing the screw press method.

6. Added value of the transnational cooperation in relation to the subject

ProRefine had a high level of transnational cooperation. In all work packages, partners cooperated across borders. We had chosen vertical and horizontal structures to build national and transnational competency in all participating six countries. The vertical structures connected scientists, industry partners and stakeholders in each region, in stakeholder group meetings and by using a participatory approach. Vertical structures aimed to facilitate creating regional knowledge and added value at national level. The horizontal structures consisted of the transnational consortium and was supported by project meetings and other communication across borders.

We developed procedures for data collection in field experiments (WP2 and WP3) that were used in different countries. Common procedures increased the value of the collected data and at the same time it was a learning process. The plot experiments in WP2 and WP3 required an experimental harvesting machine that was developed and built by project partner Trust'ing – Alf'ing in the beginning of the project. They were used successfully under different conditions and in different crops. Even here exchange of problems and solutions was a learning experience. Processing and sampling followed common procedures and most chemical analyses and assessment of digestibility were carried out at UNICATT. This eliminated the laboratory factor and made samples from different countries comparable. However, shipping frozen biological samples across borders can be a logistic challenge and pandemic restrictions made it even more difficult. We experienced that transnational cooperation from planning to chemical analyses can be a slow process, but the benefits obvious in the data analysis and writing phase.

In WP5, we developed a stronger transnational cooperation than foreseen. For better comparison and because a biorefinery plant was available at Aarhus University, we produced all experimental feeds for the pig and lamb experiments in Denmark. Thereafter the feeds were shipped to INRAE and UNICATT where the feeding experiments were done.

In WP6 we had transnational cooperation between Norway and Denmark when developing the interview guide for farmer interviews and cooperation between all participating countries when planning and analysing focus group interviews.

The ProRefine project would not have been possible without transnational cooperation. The French partner Trust'ing – Alf'ing gave us access to necessary technology and knowledge to include leaf stripping as an experimental treatment. The Danish partner AU had equipment for biorefinery and long experience in research in the field. Applying technical methods in different countries has practical challenges but increased the reliability of the results.

In addition to transnational cooperation, we also had interdisciplinary cooperation for example when carrying out stakeholder group meetings where the same questions were asked in all countries.
The project had also cooperation with other CORE Organic Cofund project. UNICATT used inoculants for fermentation experiments provided by SusOrgPlus. ProRefine and GrazyDaiSy organised a common stakeholder group meeting in Norway.

7. Suggestions for future research

Future research within fractionation and biorefinery of forages could focus on the following areas:

- Lucerne varieties and inoculation for the Nordic countries
- Using spectral instruments to assess the forage quality of forages and forage fractions
- Feeding studies with different leaf silages in pigs and poultry
- Mobile, decentralised, and cost efficient biorefinery technology
- Evaluate how soybean meal can be substituted with protein pastes without affecting pig growth performance
- Evaluate how to produce protein concentrates for legume forages with energy efficient processes
- Evaluate different strategies for reducing protein losses in lucerne silages